IN THE SPECIFICATION:

At page 1, line 3, please insert the following new paragraph:

This application is a continuation of application Serial No. 09/090,754, filed June 4, 1998, which is a continuation of application Serial No. 08/527,391, filed September 13, 1995, which issued November 17, 1998, as U.S. Patent No. 5,837,251, each of which is hereby incorporated by reference in its entirety.

Please replace the paragraph beginning at page 4, line 21, which starts "The neoplastic lesion," with the following amended paragraph:

The neoplastic lesion may evolve clonally and develop an increasing capacity for invasion, growth, metastasis, and heterogeneity, especially under conditions in which the neoplastic cells escape the host's immune surveillance (Roitt, I., Brostoff, J and ~~Kale~~ Male, D., 1993, Immunology, 3rd ed., Mosby, St. Louis, pps. 17.1-17.12).

Please replace the paragraph beginning at page 7, line 9, which starts "The ethical and fiscal," with the following amended paragraph:

The ethical and fiscal constraints which require the use of animal models for most toxicology research also impose the acceptance of certain fundamental assumptions in order to estimate dose potency in humans from dose-response data in animals. Interspecies dose-response equivalence is most frequently estimated as the product of a reference species dose and a single scaling ratio based on a physiological parameter such as body weight, body surface area, maximum ~~lifespan~~ life span potential, etc. Most frequently, exposure is expressed as milligrams of dose administered in proportion to body mass in kilograms (mg kg^{-1}). Body mass is a surrogate for body volume, and therefore, the ratio milligrams per kilogram is actually concentrations in milligrams per liter (Hirshaut, Y., et al., 1969, *Cancer Res.* 29:1732-1740). The key assumptions which accompany this practice and contribute to its failure to accurately estimate equipotent exposure among various species are: i) that the biological systems involved are homogeneous, "well-stirred volumes" with specific gravity equal to 1.0; ii) that the administered compounds are instantly and homogeneously distributed throughout the total body mass; and iii) that the response of the biological systems is directly proportional only to the initial concentration of the test material in the system. As actual pharmacokinetic conditions depart from these assumptions, the utility of initial concentration scaling between species declines.

Please replace the paragraph beginning at page 9, line 5, which starts "The effectiveness of an optimal dose," with the following amended paragraph:

The effectiveness of an optimal dose of a drug used in chemotherapy and/or immunotherapy can be altered by various factors, including tumor growth kinetics, drug resistance of tumor cells, total-body tumor cell burden, toxic effects of chemotherapy and/or immunotherapy on cells and tissues other than the tumor, and distribution of chemotherapeutic agents and/or immunotherapeutic agents within the tissues of the patient. The greater the size of the primary tumor, the greater the probability that a large number of cells (drug resistant and drug sensitive) have metastasized before diagnosis and that the patient will relapse ~~after the primary~~.

Please replace the paragraph beginning at page 9, line 17, which starts "Some metastases arise," with the following amended paragraph:

Some metastases arise in certain sites in the body where resistance to chemotherapy is based on the limited tissue distribution of chemotherapeutic drugs administered in standard doses. Such sites act as sanctuaries that shield the cancer cells from drugs that are circulating in the blood; for example, there are barriers in the brain and ~~tests~~ testes that impede drug diffusion from the capillaries into the tissue. Thus, these sites may require special forms of treatment such as immunotherapy, especially since immunosuppression is characteristic of several types of neoplastic diseases.

Please replace the paragraph beginning at page 9, line 30, which starts "The methods of the invention comprise," with the following amended paragraph:

The methods of the invention comprise methods of eliciting an immune response in an individual in whom the treatment or prevention of cancer is desired by administering a composition comprising an effective amount of a complex in which the complex consists essentially of a heat shock protein (hsp) noncovalently bound to an antigenic molecule. The amounts of the complex are within ranges of effective dosages, discovered by the present inventor to be effective, and which are surprisingly smaller than those amounts predicted to be effective by extrapolation by prior art methods from dosages used in animal studies. In a preferred embodiment, the complex is autologous to the individual; that is, the complex is isolated from the cancer cells of the individual himself (*e.g.*, preferably prepared from tumor biopsies of the patient). Alternatively, the hsp and or the antigenic molecule can be isolated from the individual or from others or by recombinant production methods using a ~~closed~~ cloned hsp originally derived from the individual or from others. "Antigenic molecule" as used herein refers to the peptides with which the hsps are endogenously associated *in vivo* (*e.g.*, in precancerous or cancerous tissue), as well as exogenous antigens/immunogens (i.e., with which the hsps are not complexed *in vivo*) or antigenic/immunogenic fragments and derivatives thereof. Such exogenous antigens and

fragments and derivatives (both peptide and non-peptide) thereof for use in complexing with hsps, can be selected from among those known in the art, as well as those readily identified by standard immunoassays known in the art by detecting the ability to bind antibody or MHC molecules (antigenicity) or generate immune response (immunogenicity).

Please replace the paragraph beginning at page 11, line 28, which starts "Immunotherapy using the therapeutic," with the following amended paragraph:

Immunotherapy using the therapeutic regimens of the invention, by administering such complexes of heat shock/stress proteins noncovalently bound to antigenic molecules, can induce specific immunity to tumor cells, and leads to regression of the tumor mass. Cancers which are responsive to specific immunotherapy by the heat shock/stress proteins of the invention include but are not limited to human sarcomas and carcinomas. In a specific embodiment, hsp-antigenic molecule complexes are allogeneic to the patient; in a preferred embodiment, the ~~hsp~~ hsp-antigenic molecule complexes are autologous to (derived from) the patient to whom they are administered.

Please replace the paragraph beginning at page 12, line 23, which starts "In addition to cancer therapy," with the following amended paragraph:

In addition to cancer therapy, the complexes of hsps noncovalently bound to antigenic molecules can be utilized for the prevention of a variety of cancers, *e.g.*, in individuals who are predisposed as a result of familial history or in individuals with an enhanced risk ~~to~~ of cancer due to environmental factors.

Please replace the paragraph beginning at page 13, line 24, which starts "Figure 3. Vaccination with cognate," with the following amended paragraph:

Figure 3. Vaccination with cognate gp96 preparations elicits MHC class I - restricted CTLs. Mice were immunized with gp96 derived from UV6138 (triangles) or UV6139SJ (rectangles) or with intact tumor cells (circles)~~, as described in legend to Fig. 1~~. Ten days after second immunization, spleens were removed and spleen cells were cocultered in a mixed lymphocyte tumor culture (MLTC) with irradiated tumor cells used for immunization or gp96 preparation. MLTCs were tested for cytotoxicity in a chromium release assay. Open symbols refer to the cytotoxicity in presence of anti-MHC class I specific antibody K44. (A) *In vitro* cytotoxicity of non-immunized mice (triangle) or mice immunized with 20 microgram of gp96 derived from UV6138 (circle) or UV6139SJ (rectangle) against targets as indicated. (B) *In vitro* cytotoxicity of non-immunized mice

(triangle) or mice immunized twice with 10^7 irradiated UV6138 cells (circles) or UV6139SJ cells (rectangles) against targets as indicated.

Please replace the paragraph beginning at page 14, line 14, which starts "Figure 5. A. ADP-bound and ADP eluted," with the following amended paragraph:

~~Figure 5. A. ADP-bound and ADP~~ FIGS. 5A-B. FIG. 5A: ATP-bound and ATP eluted hsp70 preparation was found not to be associated with peptides. ~~B. ATP-bound and ATP~~ FIG. 5B: ADP-bound and ADP eluted hsp70 preparation was found ~~not~~ to be associated with peptides.

Please replace the paragraph beginning at page 14, line 33, which starts "The methods of the invention comprise," with the following amended paragraph:

The methods of the invention comprise methods of eliciting an immune response in an individual in whom the treatment or prevention of cancer is desired by administering a composition comprising an effective amount of a complex in which the complex consists essentially of a heat shock protein (hsp) noncovalently bound to an antigenic molecule. The amounts of the complex are within ranges of effective dosages, discovered by the present inventor to be effective, and which are surprisingly smaller than those amounts predicted to be effective by extrapolation by prior art methods from dosages used in animal studies. In a preferred embodiment, the complex is autologous to the individual; that is, the complex is isolated from the cancer cells of the individual himself (*e.g.*, preferably prepared from tumor biopsies of the patient). Alternatively, the hsp and or the antigenic molecule can be isolated from the individual or from others or by recombinant production methods using a closed hsp originally derived from the individual or from others. "Antigenic molecule" as used herein refers to the peptides with which the hsps are endogenously associated *in vivo* (*e.g.*, in precancerous or cancerous tissue), as well as exogenous antigens/immunogens (i.e., with which the hsps are not complexed *in vivo*) or antigenic/immunogenic fragments and derivatives thereof. Such exogenous antigens and fragments and derivatives (both peptide and non-peptide) thereof for use in complexing with hsps, can be selected from among those known in the art, as well as those readily identified by standard immunoassays ~~know~~ known in the art by the ability to bind antibody or MHC molecules (antigenicity) or generate immune response (immunogenicity). Complexes of hsps and antigenic molecules can be isolated from cancer or precancerous tissue of a patient, or from a cancer cell line, or can be produced *in vitro* (as is necessary in the embodiment in which an exogenous antigen is used as the antigenic molecule).

At page 16, line 25, insert the following text:

TABLE 1 Enzymes and chaperones that may be involved in protein folding and assembly in cells

Organism/organelle	Enzymes			Chaperones		
Protein family	PDI	Cyclophilin PPIase	FKBP PPIase	Hsp60 (Chaperonin-60)	Hsp70 (Stress-70)	Hsp90 (Stress-90)
E. coli						
Cytosol	Thioredoxin	PPIase b		GroEL	DnaK	HtpG (C62.5)
Periplasm		PPIase a (Rotamase)				
Yeast						
Cytosol		Cph1p (Cpr1p)	Fkb1p (Fkr1p) (Rbp1p)		Ssa1-4p	Hsp83 Hsc83
ER	PDI Eug1p	yCyPB			Kar2p (BiP)	
Mitochondria				Hsp60 (Mif4p)	Ssc1p	
Drosophila						
Cytosol		CyP			Hsp68 Hsp70 Hsc1,2,4	Hsp83
ER	PDI	NinaA				
Mammals						
Cytosol		Cyclophilin (PPIase) (CyPA)	FKBP		Hsp70(p73) Hsc70(p72) (CUATpase) (Prp73)	Hsp90 (Hsp83) (Hsp87)
ER	PDI (ERp59) GSBP ERp72 ERp61	CyPB (rCyPLP)			BiP (Grp78)	Grp94 (ERp99) (endoplasmin)
Mitochondria				Hsp60 (Hsp58)	Hsp70 (Grp75)	
Plants						
Cytosol						
ER	PDI				b70(BiP)	
Chloroplasts				RuSBP		

Six protein families have been identified whose members include enzymes or chaperones proposed to be involved in folding, assembly, rearrangement or degradation of proteins in cells. Members that have been characterized to date from a variety of different organisms are shown. Alternative names are shown in parenthesis.

The *S. cerevisiae* genome contains at least nine genes related to *HSP70* of higher eucaryotes. Eight of these genes, originally named *TG100-YG107*, have been renamed on the basis of structural and functional similarities: SSA1-4 (stress seventy family *A*; *YG100*, *YG102*, *YG106*, *YG107*, respectively); *SSB1* AND *SSB2* (YG101 and YG103, respectively); *SSC1* (*YG104*); and *SSD1* (*YG105*).

Please replace the paragraph beginning at page 16, line 25, which starts "The major hsps can accumulate," with the following amended paragraph:

The major hsps can accumulate to very high levels in stressed cells, but they occur at low to moderate levels in cells that have not been stressed. For example, the highly inducible mammalian hsp70 is hardly detectable at normal temperatures but becomes one of the most actively synthesized proteins in the cell upon heat shock (Welch, et al., 1985, *J. CelinesBiol.* 101:1198-1211). In contrast, hsp90 and hsp60 proteins are abundant at normal temperatures in most, but not all, mammalian cells and are further induced by heat (Lai, et al.,

1984, *Mol. CelinesBiol.* 4:2802-10; van Bergen en Henegouwen, et al., 1987, *Genes Dev.* 1:525-31).

Please replace the paragraph at page 21, line 21, which consists of the phrase "Table 1," with the following amended paragraph:

~~Table 1~~ Table 2

Please replace the paragraph beginning at page 28, line 35, which starts "When the gp96 fraction is isolated," with the following amended paragraph:

When the gp96 fraction is isolated from the 100,000g pellet, the pellet is suspended in 5 volumes of PBS containing either 1% sodium deoxycholate or 1% ~~oxtyl~~ octyl glucopyranoside (but without the Mg^{2+} and Ca^{2+}) and incubated on ice for 1 hour. The suspension is centrifuged at 20,000g for 30 minutes and the resulting supernatant dialyzed against several changes of PBS (also without the Mg^{2+} and Ca^{2+}) to remove the detergent. The dialysate is centrifuged at 100,000g for 90 minutes, the supernatant harvested, and calcium and magnesium are added to the supernatant to give final concentrations of 2mM, respectively. Then the sample is purified by either the unmodified or the modified method for isolating gp96-peptide complex from the 100,000g supernatant, see above.

Please replace the paragraph beginning at page 30, line 1, which starts "Similarly, it has been found," with the following amended paragraph:

Similarly, it has been found that potentially immunogenic peptides may be eluted from MHC-peptide complexes using techniques well ~~know~~ known in the art (Falk, K. et al., 1990 *Nature* 348:248-251; Elliott, T., et al., 1990, *Nature* 348:195-197; Falk, K., et al., 1991, *Nature* 351:290-296).

Please replace the paragraph beginning at page 30, line 20, which starts "Briefly the complex of interest," with the following amended paragraph:

Briefly the complex of interest is centrifuged through a Centricon 10 assembly (Millipore) to remove any low molecular weight material loosely associated with the complex. The large molecular weight fraction may be removed and analyzed by SDS-PAGE while the low molecular weight may be analyzed by HPLC as described below. In the ATP incubation protocol, the stress protein-peptide complex in the large molecular weight fraction is incubated with 10mM ATP for 30 minutes at room temperature. In the low pH protocol, acetic acid or ~~trifluoro acetic~~ trifluoroacetic acid is added to the stress protein-peptide complex to give a final concentration of 10% (vol/vol) and the mixture incubated at room

temperature or in a boiling water bath or any temperature in between, for 10 minutes (*See*, Van Bleek, et al., 1990, *Nature* 348:213-216; and Li, et al., 1993, *EMBO Journal* 12:3143-3151).

Please replace the paragraph beginning at page 31, line 4, which starts "The resulting lower molecular weight fractions," with the following amended paragraph:

The resulting lower molecular weight fractions are pooled, concentrated by evaporation and dissolved in 0.1% trifluoroacetic acid (TFA). The dissolved material is then fractionated by reverse phase high pressure liquid chromatography (HPLC) ~~T~~ using for example a ~~VYDAC~~ VYDAC™ (Separations Group, Inc., Hesperia, CA) CIB reverse phase column equilibrated with 0.1% TFA. The bound material is then eluted at a flow rate of about 0.8 ml/min by developing the column with a linear gradient of 0 to 80% acetonitrile in 0.1% TFA. The elution of the peptides can be monitored by OD_{210} and the fractions containing the peptides collected.

Please replace the paragraph beginning at page 36, line 25, which starts "In an alternative embodiment," with the following amended paragraph:

In an alternative embodiment of the invention, preferred for producing complexes of hsp70 to exogenous antigenic molecules such as proteins, 5-10 micrograms of purified hsp is incubated with equimolar quantities of the antigenic molecule in 20mM sodium phosphate buffer pH 7.5, 0.5M NaCl, 3mM $MgCl_2$ and 1mM ADP in a volume of 100 microliter at 37°C for 1 hr, thereby forming an ADP-hsp-antigenic molecule complex. This incubation mixture is further diluted to 1ml in phosphate-buffered saline.

Please replace the paragraph beginning at page 37, line 6, which starts "Following complexing," with the following amended paragraph:

Following complexing, the immunogenic stress protein-antigenic molecule complexes can optionally be assayed *in vitro* using for example the mixed lymphocyte ~~target~~ tumor cell assay (MLTC) described below. Once immunogenic complexes have been isolated they can be optionally characterized further in animal models using the preferred administration protocols and excipients discussed below.

Please replace the paragraph beginning at page 37, line 16, which starts "The purified stress protein-antigenic molecule," with the following amended paragraph:

The purified stress protein-antigenic molecule complexes can be assayed for immunogenicity using the mixed lymphocyte ~~target~~ tumor culture assay (MLTC) well known in the art.

Please replace the paragraph beginning at page 38, line 6, which starts "Six days later," with the following amended paragraph:

Six days later the cultures are tested for cytotoxicity in a 4 hour ^{51}Cr-release assay (*See*, Palladino, et al., 1987, *Cancer Res.* 47:5074-5079 and Blachere, at al., 1993, *J. Immunotherapy* 14:352-356). In this assay, the mixed lymphocyte culture is added to a target cell suspension to give different effector:target (E:T) ratios (usually 1:1 to 40:1). The target cells are prelabelled by incubating $1x10^6$ target cells in culture medium containing 200 mCi ^{51}Cr/ml for one hour at 37°C. The cells are washed three times following labeling. Each assay point (E:T ratio) is performed in triplicate and the appropriate controls incorporated to measure spontaneous ^{51}Cr release (no lymphocytes added to assay) and 100% release (cells lysed with detergent). After incubating the cell mixtures for 4 hours, the cells are ~~paletted~~ pelleted by centrifugation at 2OOg for 5 minutes. The amount of ^{51}Cr released into the supernatant is measured by a gamma counter. The percent cytotoxicity is measured as cpm in the test sample minus spontaneously released cpm divided by the total detergent released cpm minus spontaneously released cpm.

Please replace the paragraph beginning at page 42, line 24, which starts "Infectious diseases that can be treated," with the following amended paragraph:

Infectious diseases that can be treated or prevented by the methods of the present invention are caused by infectious agents including, but not limited to, viruses, bacteria, fungi, protozoa and parasites.

Please replace the paragraph beginning at page 42, line 4, which starts "Radical resection offers," with the following amended paragraph:

Radical resection offers the only hope for cure in patients with hepatocellular carcinoma. Such operative procedures are associated with five-year survival rates of 12 to 30 percent. Liver transplantation may improve survival of some younger individuals. However, most patients are not surgical candidates because of extensive cirrhosis multifocal tumor pattern or scarcity of compatible donor organs. Chemotherapeutic agents have been administered either by intravenous route or through an intrahepatic arterial catheter. Such therapy has sometimes been combined with irradiation to the liver. Reductions in the size of measurable tumors of 50% or more have been reported in some patients treated with either

systemic doxorubicin or 5-fluorouracil. However, chemotherapy often induces immunosuppression and rarely causes the tumor to disappear completely and the duration of response is short. The prognosis for patients with hepatocellular carcinoma is negatively correlated with cirrhosis and metastases to the lungs or bone. Median survival for patients is only four to six months. In another specific embodiment, the present invention provides compositions and methods for enhancing specific immunity in individuals suffering from hepatocellular carcinoma in order to inhibit the progression of the neoplastic disease and ultimately ~~irradiate~~ eradicate all preneoplastic and neoplastic cells.

Please replace the paragraph beginning at page 48, line 26, which starts "The specific immunogenicity," with the following amended paragraph:

The specific immunogenicity of hsps derives not from hsps per se, but from the peptides bound to them. In a preferred embodiment of the invention directed to the use of autologous complexes of hsp-peptides as cancer vaccines, two of the most intractable hurdles to cancer immunotherapy are circumvented. First is the possibility that human cancers, like cancers of experimental animals, are antigenically distinct. In an embodiment of the present invention, hsps chaperone antigenic peptides of the cancer cells from which they are derived and circumvent this hurdle. Second, most current approaches to cancer immunotherapy focus on determining the CTL-recognized epitopes of cancer cell lines. This approach requires the availability of cell lines and CTLs against cancers. These reagents are unavailable for an overwhelming proportion of human cancers. In an embodiment of the present invention directed to autologous complexes of ~~hsp~~ hsps and peptides, cancer immunotherapy does not depend on the availability of cell lines or CTLs nor does it require definition of the antigenic epitopes of cancer cells. These advantages make autologous hsps noncovalently bound to peptide complexes attractive and novel immunogens against cancer.

Please replace the paragraph beginning at page 50, line 22, which starts "Proper technique of skin testing," with the following amended paragraph:

Proper technique of skin testing requires that the antigens be stored sterile at 4°C, protected from light and reconstituted ~~shorted~~ shortly before use. A 25- or 27-gauge ~~need~~ needle ensures intradermal, rather than subcutaneous, administration of antigen. Twenty-four and 48 hours after intradermal administration of the antigen, the largest dimensions of both erythema and induration are measured with a ruler. Hypoactivity to any given antigen or group of antigens is confirmed by testing with higher concentrations of antigen or, in ambiguous circumstances, by a repeat test with an intermediate ~~test~~ concentration.

Please replace the paragraph beginning at page 51, line 3, which starts "8 x 10^6 Peripheral blood," with the following amended paragraph:

$8x10^6$ Peripheral blood derived T lymphocytes isolated by the Ficoll-Hypaque ~~centrifigation~~ centrifugation gradient technique, are restimulated with $4x10^4$ mitomycin C treated tumor cells in 3ml RPMI medium containing 10% fetal calf serum. In some experiments, 33% secondary mixed lymphocyte culture supernatant or IL-2, is included in the culture medium as a source of T cell growth factors.

Please replace the paragraph beginning at page 53, line 8, which starts "a) Tumor Models," with the following amended paragraph:

a) Tumor models:

Two UV-induced carcinomas were studied in the C3H/HeN mice (Ward, et al., 1989, *J. Exp. Med.* 170:217): (i) the highly immunogenic ~~6138~~ UV6138 carcinoma, and (ii) the less immunogenic ~~6139ST~~ UV6139SJ carcinoma.

Please replace the paragraph beginning at page 53, line 13, which starts "b) Gp96 preparations were prepared," with the following amended paragraph:

b) Gp96 preparations were prepared from the ~~6138~~ UV6138 and ~~6139SJ~~ UV6139SJ carcinomas by the procedures described above in Section 5.2.3. The gp96 preparations were administered without adjuvants.

Please replace the paragraph beginning at page 54, line 1, which starts "Administration of gp96 isolated from," with the following amended paragraph:

Administration of gp96 isolated from the UV6138 carcinoma rendered the mice immune to the UV6138 challenge but not the UV6139SJ challenge (Figure 1). Conversely, administration of gp96 isolated from the UV6139SJ conferred resistance to the UV6139SJ cells but not to the UV6138 cells. The resistance rendered by the gp96 derived from the UV6138 against the UV6138 cells was much greater (6 out of 7 mice) than the resistance rendered by the gp96 derived from the ~~UV6139~~ UV6139SJ against the ~~UV6139-SJ~~ UV6139S cells (2 out of 4 mice) (Figure 1). These results indicate that administration of gp96 preparations derived from the two UV-induced carcinomas immunized syngeneic mice from the respective cancer cell type and that the resistance rendered was greater and more uniform against the more immunogenic carcinoma cells.

Please replace the paragraph beginning at page 56, line 25, which starts "The ability to elicit a memory response," with the following amended paragraph:

The ability to elicit a memory response is crucial for any vaccine and the ability of gp96 to elicit a memory T cell population was tested. A number of criteria, *i.e.*, radiation resistance, kinetics of appearance, loss of CD45RB and L-selectin lymphocyte surface antigens, were used to identify memory T response. In contrast to naive T cells (Schrek, R., 1961, *Ann. N.Y. Acad. Sci* 95:839), memory T cells are cycling cells (Mackay, C.R., et al, 1992, *Nature* 360:264) and like other cycling lymphocytes, are resistant to sub-lethal irradiation (Lowenthal, J.W., et al., 1991, *Leuc. Biol.* 49:388). Thus radiation-resistance can be used to distinguish naive resting T cells from activated effector and memory T cells. However, no known surface markers distinguish activated effector T cells from memory T cells and the two are distinguishable only by the kinetics of their appearance. Activated effector T cells disappear from circulation within seven to ten days of depletion of significant quantities of antigen (Sprent, J., 1994, *Cell* 76:315); in contrast, memory T cells continue to circulate well beyond this window of time. In order to test, if vaccination with tumor-derived gp96 elicits a memory T cell response in addition to the effector response shown in Fig. 3, mice were vaccinated twice at ten day intervals, with tumor-derived gp96 and were irradiated (400 rad) twelve days after the last vaccination. Three days after irradiation, MLTCs were generated from spleens of mice and tested for tumor-specific CTL response. It was observed (Fig. 4) that similar to the response in unirradiated mice (Fig. 3A), the irradiated, gp96-vaccinated mice generated powerful, MHC class I - restricted and tumor-specific CTL responses. Under this regimen of vaccination and irradiation, the irradiation eliminates the non-memory resting T cells, while the delay between the last vaccination and generation of ~~MCTCs~~ MLTCs eliminates activated T lymphocytes (Sprent, J., 1994, *Cell* 76:315). Thus, the observed CTL response derives from radiation-resistant memory T cells elicited by gp96 preparations. This phenomenon was also tested in tumor rejection assays *in vivo* and mice vaccinated with pg96 and irradiated were observed to resist tumor challenges up to 17 days after vaccination, even though they had ~~bene~~ been irradiated (data not shown). These observations indicate that vaccination with gp96 elicits a long-lived, radiation-resistant T cell population.

Please replace the paragraph beginning at page 58, line 24, which starts "The therapeutic regiment," with the following amended paragraph:

The therapeutic ~~regiment~~ regimen of hsp-peptide complexes, for example, gp96, hsp90, hsp70 or a combination thereof, includes weekly injections of the hsp-peptide complex, dissolved in saline or other physiologically compatible solution.

Please replace the paragraph beginning at page 59, line 7, which starts "Overall, the first four to six," with the following amended paragraph:

Overall, the first four to six injections are given at weekly intervals. Subsequently, two injections are given at two-week intervals; followed by a regimen of injections at monthly intervals. The effect of hsp-peptide complexes therapy is monitored by measuring: a) delayed hypersensitivity as an assessment of cellular immunity; b) activity of cytolytic T-lymphocytes *in vitro*; c) levels of tumor specific antigens, *e.g.*, carcinoembryonic (CEA) antigens; d) changes in the morphology of tumors using techniques such as a computed tomographic (CT) scan; and ~~eθ~~ e) changes in putative biomarkers of risk for a particular cancer in individuals at high risk.

Please replace the paragraph beginning at page 60, line 13, which starts "Meth A sarcoma cells," with the following amended paragraph:

Meth A sarcoma cells (500 million cells) were homogenized in hypotonic buffer and the lysate was centrifuged at 100,000 g for 90 minutes at 4°C. The supernatant was divided into two and was applied to an ADP-agarose or an ATP-agarose column. The columns were washed in buffer and were eluted with 3 mM ADP or 3 mM ATP, respectively. The eluted fractions were analyzed by SDS-PAGE: in both cases, apparently homogeneous preparations of hsp70 were obtained. However, when each of the preparations was tested for presence of peptides, the ADP-bound/eluted hsp70 preparation was found to be associated with peptides (*i.e.*, the hsp70 preparation was an ADP-hsp70-peptide complex), while the ATP-bound/eluted hsp70 preparation was not. ~~(Figures. 5A and 5B)~~ (Figures 5B and 5A, respectively)